

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>5</sup> :</b> C12N 5/10, 15/09, 15/11 C12N 15/12, 15/03, 15/07 G01N 33/566, C07K 15/06, 15/14 C07K 15/24, 15/28	<b>A1</b>	<b>(11) International Publication Number:</b> WO 92/12236  <b>(43) International Publication Date:</b> 23 July 1992 (23.07.92)
<b>(21) International Application Number:</b> PCT/US91/00236 <b>(22) International Filing Date:</b> 11 January 1991 (11.01.91)  <b>(71) Applicants:</b> THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 300 Lakeside Drive, 22nd Floor, Oakland, CA 94612-3550 (US). SCRIPPS CLINIC AND RESEARCH CENTER [US/US]; 10666 North Torrey Pines Road, La Jolla, CA 92037 (US).  <b>(72) Inventors:</b> SHEPPARD, Dean ; 1006 Hubert Road, Oakland, CA 94610 (US). QUARANTA, Vito ; 8861 Nottingham Place, La Jolla, CA 92037 (US). PYTELA, Robert ; 22 Flint Street, San Francisco, CA 94114 (US).		<b>(74) Agents:</b> CAMPBELL, Cathryn et al.; Pretty, Schroeder, Brueggemann & Clark, 444 South Flower Street, Suite 2000, Los Angeles, CA 90071 (US).  <b>(81) Designated States:</b> CA, JP.  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> A NOVEL INTEGRIN $\beta$ SUBUNIT AND USES THEREOF  <b>(57) Abstract</b>  The present invention provides substantially pure integrins containing a novel $\beta$ subunit designated as $\beta_6$ . The novel $\beta_6$ subunit forms heterodimers with $\alpha_V$ and $\alpha_F$ . Methods of controlling cell adhesion using the $\beta_6$ -containing integrins are also provided.		

44

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MN	Mongolia
BE	Belgium	GA	Gabon	MR	Mauritania
BF	Burkina Faso	GB	United Kingdom	MW	Malawi
BG	Bulgaria	GN	Guinea	NL	Netherlands
BJ	Benin	GR	Greece	NO	Norway
BR	Brazil	HU	Hungary	PL	Poland
CA	Canada	IT	Italy	RO	Romania
CF	Central African Republic	JP	Japan	RU	Russian Federation
CG	Congo	KP	Democratic People's Republic of Korea	SD	Sudan
CH	Switzerland	KR	Republic of Korea	SE	Sweden
CI	Côte d'Ivoire	LI	Liechtenstein	SN	Senegal
CM	Cameroon	LK	Sri Lanka	SU	Soviet Union
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
DE	Germany	MC	Monaco	TG	Togo
DK	Denmark			US	United States of America

A NOVEL INTEGRIN  $\beta$  SUBUNIT  
AND USES THEREOF

This work was supported in part by research grants HL/AL 33259, CA-47541 and CA-47858 from the  
5 National Institutes of Health. The U.S. Government has rights in the invention.

TECHNICAL FIELD

This invention relates to receptors for adhesion peptides, and more specifically to a novel  
10 receptor subunit having affinity for extracellular matrix molecules.

BACKGROUND ART

Multicellular organisms, such as man, have some  $10^{14}$  cells which can be divided into a minimum of fifty  
15 different types, such as blood cells and nerve cells. During the course of growth and development, cells adhere to other cells, or to extracellular materials, in specific and orderly ways. Such cell adhesion mechanisms appear to be of importance in mediating patterns of  
20 cellular growth, migration and differentiation, whereby cells develop specialized characteristics so as to function as, for example, muscle cells or liver cells. Cell adhesion mechanisms are also implicated in dedifferentiation and invasion, notably where cells lose  
25 their specialized forms and become metastasizing cancer cells.

The mechanisms underlying the interactions of cells with one another and with extracellular matrices are not fully understood, but it is thought that they are  
30 mediated by cell surface receptors which specifically recognize and bind to a cognate ligand on the surface of cells or in the extracellular matrix.

The adhesion of cells to extracellular matrices and their migration on the matrices is mediated in many cases by the binding of a cell surface receptor to an Arg-Gly-Asp containing sequence in the matrix protein, as reviewed in Ruoslahti and Pierschbacher, Science 238:491 (1987). The Arg-Gly-Asp sequence is a cell attachment site at least in fibronectin, vitronectin, fibrinogen von Willibrand, thrombospondin, osteopontin, and possibly various collagens, laminin and tenascin. Despite the similarity of their cell attachment sites, these proteins can be recognized individually by their interactions with specific receptors.

The integrins are a large family of cell surface glycoproteins that mediate cell-to-cell and cell-to-matrix adhesion as described, for example, in the Ruoslahti and Pierschbacher article cited above. All known members of this family of adhesion receptors are heterodimers consisting of an  $\alpha$  and a  $\beta$  subunit noncovalently bound to each other. When the integrin family was first identified, integrins were grouped into three subfamilies based on the three  $\beta$  subunits that were initially recognized ( $\beta_1$ ,  $\beta_2$  and  $\beta_3$ ). Over the past few years, the primary structures of three integrin  $\beta$  subunits from mammalian cells and one from Drosophila have been deduced from cDNA.

Each  $\alpha$  subunit was thought to associate uniquely with a single  $\beta$  subunit. Eleven distinct  $\alpha$  subunits have thus far been described. As new integrins have been identified, however, it has become clear that this grouping is not entirely satisfactory, since there are clearly more than three  $\beta$  subunits and since some  $\alpha$  subunits can associate with more than one  $\beta$  subunit as described, for example, in Sonnenberg et al., J. Biol. Chem. 265:14030-14038 (1988).

Because of the importance of integrins in mediating critical aspects of both normal and abnormal cell processes, a need exists to identify and characterize different integrins. The present invention satisfies this need and provides related advantages as well.

#### SUMMARY OF THE INVENTION

The present invention relates to a substantially purified  $\beta$  subunit of an integrin cell surface receptor designated as  $\beta_6$ . The amino acid sequence of  $\beta_6$  is provided in Figure 3.

The present invention also relates to amino acid fragments specific to  $\beta_6$  that have a variety of uses. The invention further relates to vectors having a gene encoding such fragments. Host cells containing such vectors are also provided. The nucleic acids encoding  $\beta_6$  as well as nucleic acids that specifically hybridize with the nucleic acids encoding  $\beta_6$  sequences are other aspects of the present invention.

In a further aspect, the present invention relates to a substantially purified integrin comprising  $\beta_6$  bound to an  $\alpha$  subunit, particularly  $\alpha_v$  or  $\alpha_f$ . Methods of blocking the attachment of the  $\beta_6$ -containing integrins to its ligand and of detecting the binding of such integrins to its ligand are also provided.

The present invention also relates to methods of increasing or decreasing cell adhesion in cells expressing a  $\beta_6$ -containing integrin by overexpressing the integrin or by binding the integrin with a ligand, such as vitronectin.

### BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the design of PCR primers.

Figure 2 shows a map of sequencing strategy.

Figure 3 shows the nucleotide sequence and  
5 amino acid translation for human (H) and guinea pig (GP)  
 $\beta_6$ .

Figure 4 shows the alignment of  $\beta_6$  with four  
previously reported integrin  $\beta$  subunits.

Figure 5 shows the alignment of partial  
10 nucleotide and amino acid sequences from human (H) and  
guinea pig (GP)  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$ , and  $\beta_6$  for the region just  
downstream from the B3F primer.

### DETAILED DESCRIPTION OF THE INVENTION

15 The present invention provides a composition of  
matter relating to a novel, substantially purified  
integrin  $\beta$  subunit, referred to herein as  $\beta_6$ . The amino  
acid sequence of  $\beta_6$  is also provided and is shown in  
Figure 3.

20 By "substantially purified" is meant  
substantially free of contaminants normally associated  
with a native or natural environment.

By " $\beta_6$ " is meant a polypeptide having  
substantially the same amino acid sequence and binding  
25 functions of the polypeptides encoded by the sequences  
set forth in Figure 3 for human and guinea pig  $\beta_6$ . Thus,  
modified amino acid sequences that do not substantially  
destroy the functions and retain the essential sequence  
of  $\beta_6$  are included within the definition of  $\beta_6$ . Amino

acid sequences, such as the sequence for  $\beta_1$ ,  $\beta_2$  and  $\beta_3$ , having less than 50% homology with the sequence of  $\beta_6$ , are not substantially the same sequence and, therefore, do not fall within the definition of  $\beta_6$ . Given the amino acid sequences set forth herein, additions, deletions or substitutions can be made and tested to determine their effect on the function of  $\beta_6$ . In addition, one skilled in the art would recognize that certain amino acids, such as the conserved cystines, for example, can be modified to alter a binding function of  $\beta_6$ .

Amino acids are identified herein by the standard one-letter abbreviations, as follows:

	Amino Acid	Symbol
15		
	Alanine	A
	Asparagine	N
	Aspartic acid	D
	Arginine	R
20	Cysteine	C
	Glutamine	Q
	Glutamic acid	E
	Glycine	G
	Histidine	H
25	Isoleucine	I
	Leucine	L
	Lysine	K
	Methionine	M
	Phenylalanine	F
30	Proline	P
	Serine	S
	Threonine	T
	Tryptophan	W
	Tyrosine	Y
35	Valine	V

Based on its amino acid sequence, the  $\beta$  subunit of the present invention is clearly different from  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$  and other  $\beta$  subunits that have recently been discovered. For example, the 11-amino acid carboxyl-terminal extension on  $\beta_6$  distinguishes it from  $\beta_1$ ,  $\beta_2$ , and

$\beta_3$ . The short cytoplasmic tails of  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$  are thought to be sites of interaction with the cytoskeleton and regions for the transduction of signals initiated by interactions of the large extracellular domains with ligands. These cytoplasmic tails may also be targets for regulation of integrin function. The distinctive 11-amino acid cytoplasmic tail of  $\beta_6$  indicates that its regulation or pathways for signal transduction may be different from those of  $\beta_1$ ,  $\beta_2$  and  $\beta_3$ .

10 In addition to  $\beta_1$ ,  $\beta_2$  and  $\beta_3$ , recent studies have suggested the existence of as many as five other integrin  $\beta$  subunits. A  $\beta$  subunit with a molecular weight of approximately 210,000 ( $\beta_4$ ) has been found associated with the integrin  $\alpha$  subunit " $\alpha_6$ " in colon carcinoma cells and in a variety of other tumor cells of epithelial origin as described, for example, in Kajiji et al., EMBO J., 8:673-680 (1989). On the basis of its high molecular weight, 210,000 compared with the predicted size of 106,000 of the subject novel protein, and on the basis of its clearly different amino-terminal sequence, it is apparent that  $\beta_4$  is not the same as the subject polypeptide.

Another  $\beta$  subunit, originally called  $\beta_x$  was identified in epithelial-derived tumor cells in association with the integrin  $\alpha$  subunit  $\alpha_v$  as described, for example, in Cheresch et al., Cell 57:59-69 (1989). This  $\beta$  subunit, having a distinctive amino-terminal sequence, was recently renamed  $\beta_5$ . Based on recent studies of purified preparations,  $\beta_5$  clearly differs from the  $\beta$  subunit of the present invention. Because the  $\beta$  subunit described in the present report is distinct from each of the five  $\beta$  subunits for which sequence information is available, it has been designated as  $\beta_6$ .

The existence of two other integrin  $\beta$  subunits



has been inferred from the identification of unique proteins after immunoprecipitation of surface-labeled cell lysates with antibodies to known  $\alpha$  subunits. One of these novel proteins, called  $\beta_5$ , was found in association  
5 with  $\alpha_v$  in the human osteosarcoma cell line MG-63, in the fibroblast cell line AF1523, and in human endothelial cells as described, for example, in Freed et al., EMBO J. 8:2955-2965 (1989). This subunit is also different from  $\beta_6$  since  $\beta_5$  is expressed in MG-63 cells while  $\beta_6$  is not  
10 expressed in these cells as shown in Table 1.

The other novel integrin  $\beta$  subunit identified by co-immunoprecipitation of known  $\alpha$  subunits,  $\beta_p$ , is a protein of about  $M_r$  95,000 that is found to be associated with  $\alpha_4$ , an  $\alpha$  subunit first found as part of the  
15 lymphocyte homing receptor VLA-4 as described, for example, in Holzmann et al., Cell 45:37-46 (1989). This subunit is also distinct from  $\beta_6$  since  $\beta_p$  is expressed in lymphocytes while  $\beta_6$  is not expressed in lymphocytes as shown in Table 1.

TABLE 1  
Distribution of B<sub>6</sub>

	<u>Type</u>	<u>Results</u>	<u>Source</u>
<u>Cell Lines:</u>			
5 FG-2	Pancreatic	+	Kajiji et al. EMBO J 3:673-80 (1989)
Panc I	Pancreatic	-	Dr. Metzgar, Duke U., N.C.
Colo-396	Colon CA	+	Dr. L. Walker, Cytel, San Diego, CA
UCLA P3	Lung CA	+	Dr. L. Walker, Cytel, San Diego, CA
Hela	Cervical	-	ATCC #CCL-2
10 Jar	Chorio CA	+	ATCC #HTB 36
HT 1080	Fibrosarcoma	-	ATCC #CCL 121
U 937	Monocytoid	-	ATCC #CRL 1593
M 21	Melanoma	-	Dr. R. Reisfeld, Scripps Clinic & Research Foundation, La Jolla, CA
B 16	Melanoma	-	Dr. R. Reisfeld Scripps Clinic & Research Foundation, La Jolla, CA
15 MG 63	Osteosarcoma	-	ATCC #CRL 1427
<u>Tissues:</u>			
	Cervix	+	
	Aortic Endothelium	-	
	Leukocytes	-	

The invention also provides an integrin comprising  $\beta_6$  bound to an  $\alpha$  subunit.  $\beta_6$ , consistent with recent findings of other  $\beta$  subunits, can associate with a variety of  $\alpha$  subunits to form a functional integrin. In one embodiment,  $\beta_6$  associates with  $\alpha_v$ . In another embodiment,  $\beta_6$  associates with another  $\alpha$  subunit referred to herein as  $\alpha_f$ . The  $\alpha_v \beta_6$  integrin, as well as other integrins containing  $\beta_6$ , can bind molecules, for example extracellular matrix molecules. Such molecules are referred to herein as ligands. In a specific embodiment, certain  $\beta_6$ -containing integrins can bind Arg-Gly-Asp-containing polypeptides such as vitronectin or fibronectin. The binding of  $\beta_6$ -containing integrins to various ligands can be determined according to procedures known in the art and as described for example, in Ruoslahti & Pierschbacher, Science 238:491-497 (1987).

The invention also provides an amino acid fragment specific to  $\beta_6$ . Since  $\beta_6$  is a novel molecule, it contains many fragments which are specific for this  $\beta$  subunit. Fragments specific to  $\beta_6$  contain sequences having less than 50% homology with sequences of other known integrin  $\beta$  subunit fragments. These fragments are necessarily of sufficient length to be distinguishable from known fragments and, therefore, are "specific for  $\beta_6$ ." The amino acid sequence of such fragments can readily be determined by referring to the figures which identify the  $\beta_6$  amino acid sequences. These fragments also retain the binding function of the  $\beta_6$  subunit and can therefore be used, for example, as immunogens to prepare reagents specific for  $\beta_6$  or as an indicator to detect the novel  $\beta_6$ -containing integrin of the present invention. One skilled in the art would know of other uses for such fragments.

The invention also provides a reagent having specificity for an amino acid sequence specific for  $\beta_6$ .

Since  $\beta_6$  is a novel protein with at least 50% amino acid differences over related  $\beta$  subunits, one skilled in the art could readily make reagents, such as antibodies, which are specifically reactive with amino acid sequences specific for  $\beta_6$  and thereby immunologically distinguish  $\beta_6$  from other molecules. Various methods of making such antibodies are well established and are described, for example, in Antibodies, A Laboratory Manual, E. Harlow and D. Lane, Cold Spring Harbor Laboratory 1988, pp. 139-283 and Huse et al., Science 24:1275-1280 (1988).

The invention also provides nucleic acids which encode  $\beta_6$ . Examples of such sequences are set forth in Figure 3. Following standard methods as described, for example, in Maniatis et al., Molecular Cloning, Cold Spring Harbor (1982), nucleic acid sequences can be cloned into the appropriate expression vector. The vector can then be inserted into a host, which will then be capable of expressing recombinant proteins. Thus, the invention also relates to vectors containing nucleic acids encoding such sequences and to hosts containing these vectors.

The sequences set forth in Figure 3 also provide nucleic acids that can be used as probes for diagnostic purposes. Such nucleic acids can hybridize with a nucleic acid having a nucleotide sequence specific for  $\beta_6$  but do not hybridize with nucleic acids encoding non- $\beta_6$  proteins, particularly other cell surface receptors. These nucleic acids can readily be determined from the sequence of  $\beta_6$  and synthesized using a standard nucleic acid synthesizer. Nucleic acids are also provided which specifically hybridize to either the coding or non-coding DNA of  $\beta_6$ .

Integrin cell surface receptors bind ligands, such as extracellular matrix molecules. However, the

binding of the integrin to the ligand can be blocked by various means. For example, the binding of a  $\beta_6$ -containing integrin can be blocked by a reagent that binds the  $\beta_6$  subunit or the  $\beta_6$ -containing integrin.

- 5 Examples of such reagents include, for example, Arg-Gly-Asp-containing peptides and polypeptides, ligand fragments containing the integrin binding site, as well as antibodies specifically reactive with  $\beta_6$  or a  $\beta_6$ -containing integrin. Alternatively, the blocking can be
- 10 carried out by binding the ligand or fragment thereof, recognized by a  $\beta_6$ -containing integrin with a reagent specific for the ligand at a site that inhibits the ligand from binding with the integrin. Since the binding of a  $\beta_6$ -containing integrin to its ligand can mediate cell
- 15 adhesion to an extracellular matrix molecule, preventing this binding can prevent cell adhesion. Alternatively, cell adhesion can be promoted by increasing the expression of  $\beta_6$ -containing integrins by a cell.

- Finally, the invention provides a method of
- 20 detecting ligands which bind a  $\beta_6$ -containing integrin. The method comprises contacting a  $\beta_6$ -containing integrin with a solution containing ligands suspected of binding  $\beta_6$ -containing integrins. The presence of ligands which bind a  $\beta_6$ -containing integrin is then detected.

- 25 In summary, the invention claims:

1. A substantially purified integrin cell surface receptor subunit comprising  $\beta_6$ .
2. The substantially purified integrin cell surface receptor subunit of claim 1 having the amino acid
- 30 sequence set forth in Figure 3 for human.
3. A substantially purified integrin comprising  $\beta_6$  bound to an  $\alpha$  subunit.

4. The integrin of claim 3, wherein the subunit is  $\alpha_v$ .
5. The integrin of claim 3, wherein the subunit is  $\alpha_f$ .
- 5 6. A substantially purified amino acid fragment specific to  $\beta_6$ .
7. A vector comprising a gene encoding for the amino acid fragment of claim 6.
8. A host containing the vector of claim 7.
- 10 9. A reagent having specificity for an amino acid sequence specific for  $\beta_6$ .
10. The reagent of claim 9, wherein the reagent is an antibody.
11. A substantially purified nucleic acid  
15 encoding  $\beta_6$ .
12. A substantially purified nucleic acid which specifically hybridizes with a nucleotide sequence of the nucleic acid of claim 11.
13. A substantially purified nucleic acid  
20 which specifically hybridizes with the nucleic acid of claim 12 and does not hybridize with a nucleic acid encoding a non- $\beta_6$  polypeptide.

14. A method of preventing the binding of a cell expressing a  $\beta_6$ -containing integrin to ligand capable of binding to said  $\beta_6$ -containing integrin, comprising blocking the binding of the  $\beta_6$ -containing integrin and the  
5 ligand.

15. The method of claim 14, wherein the blocking is effected by binding the  $\beta_6$ -containing integrin with a reagent specific thereto.

16. The method of claim 14, wherein the  
10 blocking is effected by binding the ligand of the  $\beta_6$ -containing integrin with a reagent specific for the ligand.

17. The method of claim 15, wherein the reagent is an RGD-containing peptide or polypeptide.

18. The method of claim 15, wherein the  
15 reagent is a ligand fragment containing an integrin binding site.

19. A method of detecting a ligand that binds a  $\beta_6$ -containing integrin, comprising contacting the  $\beta_6$ -  
20 containing integrin with a solution containing the ligand suspected of binding  $\beta_6$ -containing integrins and detecting the presence of the ligand bound to the  $\beta_6$ -containing integrin.

20. A method of increasing cell adhesion in  
25 cells expressing a  $\beta_6$ -containing integrin, comprising overexpressing the  $\beta_6$ -containing integrin in a cell.

21. A method of decreasing cell adhesion in cells expressing a  $\beta_6$ -containing integrin comprising binding the  $\beta_6$ -containing integrin with a ligand.

The following examples are intended to illustrate but not limit the invention.

EXAMPLE I

Identification of a Novel  $\beta$  Subunit

5 Generation of cDNA Fragments by Polymerase Chain Reaction

Tracheal epithelial cells harvested from male Hartley outbred guinea pigs (Charles River Breeding Laboratories, Bar Harbor, ME) were grown to confluence over 10-14 days on collagen-impregnated microporous  
10 filters commercially available from Costar. RNA was harvested from these primary cultures, and mRNA was purified over oligo(dT)-cellulose columns using the Fast Track mRNA isolation kit (Invitrogen, San Diego, California). Two to 5  $\mu$ g of mRNA was used as a template  
15 for cDNA synthesis catalyzed by 200 units of Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, MD) in a 20-40  $\mu$ l reaction volume. One to 5  $\mu$ l of the resultant cDNA was used as a template for polymerase chain reaction (PCR).  
20 PCR was carried out in a reaction volume of 25-200  $\mu$ l. In addition to the template cDNA, each PCR reaction contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25°C), 1.5 mM  $MgCl_2$ , 0.01% gelatin, 0.1% Triton X-100, 0.2 mM each of dATP, dGTP, dCTP and dTTP, and 0.05 units/ $\mu$ l Taq DNA  
25 polymerase (obtained from either United States Biochemical Corporation, Cleveland, OH, or from Promega, Madison, WI).

For each reaction, two oligonucleotide primers were also added to obtain a final concentration of 1  $\mu$ M  
30 each. The primer pairs are identified below. Each reaction mixture was overlaid with mineral oil, heated to 95°C for 4 min. in a thermal cycler (Ericomp, San Diego, CA), and then subjected to 30 cycles of PCR. Each cycle



consisted of 45 seconds at 95°C, 45 seconds at 53°C, and 1 min. at 72°C. Immediately after the last cycle, the sample was maintained at 72°C for 10 min.

The results of each PCR reaction were analyzed  
5 by gel electrophoresis in 1.5% agarose. Reactions that produced fragments of the expected size were electrophoresed in 1.5% low gel temperature agarose (Bio-Rad Laboratories, Richmond, CA). The appropriate size band was excised, melted at 68°C, and the DNA was  
10 purified by extraction with phenol/chloroform and precipitation in ethanol and ammonium acetate.

#### PCR Primers

To obtain the initial fragment of the novel  $\beta$  subunit cDNA described herein, degenerate mixtures of PCR  
15 primers were used. Oligonucleotides were synthesized, trityl-on, by the University of California, San Francisco Biomolecular Resource Center using a DNA synthesizer with standard procedures, and purified over NEN-sorb cartridges (DuPont-New England Nuclear, Boston, MA).  
20 These consensus primer mixtures were designed to anneal with the nucleotides encoding the highly conserved sequence Asp-Leu-Tyr-Tyr-Leu-Met-Asp-Leu (primer B1F) and Glu-Gly-Gly-Asp-Ala-Ile-Met-Gln (primer B2R) that flank an approximately 300-nucleotide region beginning  
25 approximately 130 amino acids from the amino terminus of each of the integrin  $\beta$  subunits sequenced to date. The sequences of the primers identified herein are depicted in Figure 1.

On the basis of the initial sequence obtained,  
30 a specific forward primer was designed to anneal with the sequence encoding the amino acids Pro-Leu-Thr-Asn-Asp-Ala-Glu-Arg (primer BTE2F) ending approximately 49

nucleotides from the 3' end of the region we had sequenced. We also designed an additional forward primer (B3F) and two reverse primers (B3R and B4R) to recognize highly conserved consensus regions encoding the sequences  
5 Gly-Glu-Cys-Val-Cys-Gly-Gln-Cys (B3 region) and Ile-Gly-Leu-Ala-Leu-Leu-Ile-Trp-Lys (B4 region). The alignment of these primers with previously published sequences of human  $\beta_1$ ,  $\beta_2$  and  $\beta_3$  and chicken  $\beta_1$  is shown in Fig. 1. PCR as described above was performed with cDNA  
10 from guinea pig tracheal epithelial cells and the primer pairs BTE2F/B3R and B3F/B4R.

The primer pair BTE2F/B3R yielded 1095 additional base pairs of new sequence. Based on this sequence another specific primer (BTE3F) was designed to  
15 recognize the sequence Val-Ser-Glu-Asp-Gly-Val near the 3' end of this sequence, and PCR was performed with this primer in combination with primer B4R.

Figure 1 shows the design of PCR primers.  $\beta$  subunit consensus primer mixtures were designed on the  
20 basis of alignment of published sequences of human  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$  and chicken  $\beta_1$ . For forward primers (B1F and B3F), the primer sequences included a single nucleotide whenever possible for each of the first two nucleotides of each codon and were usually either degenerate or included  
25 deoxyinosine for the third base in codons for amino acids other than methionine. Reverse primers (B2R, B3R, and B4R) were designed in the same manner for the complementary DNA strand. Two specific forward primers were designed to recognize  $\beta_6$ . The first (BTE2F) was  
30 designed to work across species and was thus degenerate or included deoxyinosine in the third codon position. The second, BTE3F, was not degenerate and was designed to only recognize guinea pig  $\beta_6$ .

Cloning of Fragments Obtained by PCR

- Individual fragments were cloned in pBluescript (Stratagene, San Diego, CA) as follows. Purified fragments were resuspended in distilled water containing
- 5 deoxynucleotides and treated with 2.5 units of DNA polymerase I, large fragment (Promega) to fill in any 3' recessed ends left after the last cycle of PCR. The 5' ends were phosphorylated with 5 units of T4 polynucleotide kinase (New England Biolabs, Beverly, MA).
- 10 An aliquot of the above reaction mixture containing approximately 100-200 ng of DNA, was ligated into pBluescript that had been cut with EcoRV (Promega) and dephosphorylated with calf intestinal alkaline phosphatase (Boehringer Mannheim, Indianapolis, IN).
- 15 Ligations were performed at 22°C for 1 hour with T4 DNA ligase (Bethesda Research Laboratories). The ligation mixture was used to transform competent Escherichia coli (JM109, Clontech, San Francisco, California). Plasmids containing inserts were purified using the Pharmacia
- 20 miniprep lysis kit (Pharmacia LKB Biotechnology, Inc., Piscataway, NJ) denatured in 0.3 M NaOH, further purified over spin columns containing Sephacryl S-400 (Pharmacia), and then sequenced using the Sequenase™ version 2.0 sequencing kit (United States Biochemical Corp.,
- 25 Cleveland, OH) and [<sup>35</sup>S]dATP (Amersham Corp., Arlington Heights, IL).

Library Screening

- PCR fragments generated with the primer pairs B1F/B2R and BTE3F/B4R were uniformly labeled with alpha-
- 30 [<sup>32</sup>P]dCTP and used as probes to screen a random-primed cDNA library and an oligo-dT-primed cDNA library both constructed in the plasmid pTZ18R-BstXI (Invitrogen) from mRNA obtained from the human pancreatic carcinoma cell line FG-2. Plasmid was purified from clones found to

hybridize with either region, and inserts were sequenced. A portion of insert DNA from one clone was in turn labeled and used to screen the same libraries. Fourteen independent overlapping clones were sequenced from both ends using primers that recognize regions of the pTZ polylinker. The regions flanking the 3' end of the putative translated region of the new  $\beta$  subunit were sequenced in both directions from three clones using primers constructed to recognize sequences close to the 3' end. On the basis of the initial sequences thus obtained, an additional internal sequence was obtained from clones T10, T11, T12 and T14 (Fig. 2) after digestion with specific restriction endonucleases and relegation. Three internal fragments thus generated were subcloned into pBluescript and were also sequenced in both directions. Approximately 90% of the new sequence reported was obtained from both strands of DNA, and 97% was obtained from two or more overlapping clones (Fig. 2).

Figure 2 shows a map of the sequencing strategy. Shown are the location of clones used to obtain the partial cDNA sequence of guinea pig  $\beta_6$  (clones 1F, 3L, 3N and 3Y, top) and the complete sequence of human  $\beta_6$  (clones T1-T19 bottom). Also shown is the location of the translated region (Protein). The location of the transmembrane domain is shown by the letters TM. Clones shown often represent one of several identical clones. Internal sequence of clones with long inserts was obtained by restriction endonuclease digestion and relegation and by ligation of internal fragments into pBluescript. Specific restriction sites employed are shown (Hind, HindIII; Hinc, HincII; Kpn, KpnI; Pst, PstI). The direction and extent of sequencing are shown by arrows. 1109 and 1110 are the sites recognized by oligonucleotide sequencing primers. T18 and T19 each terminated in a poly(A) tail. The regions

recognized by the degenerate PCR primers B1F (B1), B2R (B2), B3R/F (B3), and B4R (B4) and the  $\beta_6$  primers BTE2F (BTE2) and BTE3F (BTE3) are noted above the guinea pig cDNA map, kb, kilobases.

5 Nucleotide Sequence of a Novel Guinea Pig Integrin  $\beta$  Subunit

PCR using cDNA from guinea pig airway epithelial cells and the consensus primer mixtures B1F and B2R (Fig. 1) amplified DNA fragments with the  
10 expected size of approximately 350 nucleotides. When the fragment DNA was sequenced after cloning into pBluescript, recombinant clones each contained inserts with one of two distinct sequences. One sequence encoded a stretch of 98 amino acids that was 97% identical to the  
15 expected region of human  $\beta_1$  and was therefore presumed to be guinea pig  $\beta_1$ . The other sequence encoded 98 amino acids that were only 53% identical to human  $\beta_1$ , 45% identical to human  $\beta_2$ , and 57% identical to human  $\beta_3$  (Fig. 2, clone 1F). Both of the guinea pig sequences included  
20 the integrin  $\beta$  subunit consensus sequences Ser-X-Ser-Met-X-Asp-Asp-Leu and Gly-Phe-Gly-Ser-Phe-Val, and both contained the 2 cysteine residues found in this region in all known integrin  $\beta$  subunits. These data suggest that one of the two sequences we obtained encoded a new member  
25 of the integrin  $\beta$  subunit family.

This novel sequence was extended by further PCR steps utilizing primers specific for the novel sequence (BTE2F, BTE3F) in combination with two additional degenerate primers (B3R and B4R, see Figs. 1, 2 and 4).  
30 With the primer pair BTE2F/B3R two different cDNA products were obtained (3L and 3N in Fig. 2) due to an unexpected hybridization of the B3R primer with a site 220 nucleotides further downstream (B3' in Fig. 2). The 1732-nucleotide sequence determined from these clones is

shown in Fig. 3.

Figure 3 shows Nucleotide sequence and amino acid translation for human (H) and guinea pig (GP)  $\beta_6$ . The amino acid translation is denoted by the single letter code beneath the second nucleotide of each codon from the translated region of human  $\beta_6$ . For the guinea pig sequence, only amino acids that differ from the human sequence are shown. The numbers along the right-hand margin denote the nucleotide or amino acid number of the last entry on each line. The numbering system used starts with the first nucleotide or amino acid available for each sequence shown. The nine potential sites for N-glycosylation in the putative extracellular domain of human  $\beta_6$  are underlined.

15 Nucleotide Sequence of Human  $\beta_6$

Screening of cDNA libraries constructed from the human pancreatic carcinoma cell line FG-2 with guinea pig cDNA probes 1F and 3Y (see Fig. 2) and subsequent screening with a probe constructed from a portion of clone T10 (Fig. 2) produced 14 independent positive clones. The two longest clones (T18 and T19) extended to the poly(A) tail. A map of these clones, constructed on the basis of sequence information and of the mobility of inserts cut out of these clones in agarose gels is shown in Fig. 2. This map predicts an mRNA of approximately 5 kilobases including at least a 226-nucleotide untranslated region at the 5' end and, a 2364-nucleotide open reading frame, and a 3' untranslated region of approximately 2.5 kilobases. This molecule has been termed integrin  $\beta_6$ .

Fig. 3 shows the partial nucleotide and complete amino acid sequences for human  $\beta_6$  (excluding most of the 3'-untranslated region) and the alignment of the

1732 nucleotides of sequence obtained from PCR of guinea pig airway epithelial cell cDNA. Of the 577 amino acids deduced from the region sequenced in both species only 36 residues differ; the amino acid sequences are 94% identical. Furthermore, of the 1732 nucleotides sequenced in both species, 91% are identical. Nine potential glycosylation sites present in the putative extracellular domain of human  $\beta_6$  are shown by underlining. All seven of these sites that lie within the 577 amino acids obtained for guinea pig  $\beta_6$  are also present in the guinea pig protein. If all of the potential glycosylation sites are occupied with oligosaccharides having an average molecular weight of 2,500, the predicted molecular weight of human  $\beta_6$  would be 106,000.

Comparison of the 788-amino acid sequence deduced from the open reading frame to the three previously sequenced human  $\beta$  subunits and the myospheroid protein of Drosophila is shown in Fig. 4.

Figure 4 shows the alignment of  $\beta_6$  with four previously reported integrin  $\beta$  subunits. Previously published sequences for human  $\beta_1$ , human  $\beta_2$ , human  $\beta_3$ , the myospheroid gene product ( $\beta_{myo}$ ) of Drosophila, and the novel sequence described as ( $\beta_6$ ) are shown using the single letter amino acid code. The 56 conserved cysteines are noted by \* and the 120 other invariant amino acids by = above each line. The transmembrane domain is underlined. The regions used for constructing the consensus  $\beta$  subunit primers B1F (1), B2R (B2), B3F/R (B3), and B4R (B4) are labeled below the alignment in bold type. The numbers along the right-hand margin denote the number of the last amino acid in each line beginning from the first amino acid of each putative signal sequence.

There are 179 amino acid residues that are identical in each of the other  $\beta$  subunits and in  $\beta_6$  including 56 conserved cysteine residues. The overall percentage of identical amino acids between  $\beta_6$  and the other human  $\beta$  subunits is 47% for  $\beta_3$ , 42% for  $\beta_1$ , and 38% for  $\beta_2$ . Human  $\beta_6$  is also 39% identical to the Drosophila  $\beta$  subunit. Human  $\beta_1$ ,  $\beta_2$  and  $\beta_3$  and the Drosophila  $\beta$  subunit all have cytoplasmic regions consisting of 41 amino acids (beginning after the putative transmembrane domain shown by the underline in Fig. 4). Although  $\beta_6$  contains each of the 10 conserved amino acid residues in this cytoplasmic region it also contains an 11-amino acid extension at the carboxyl terminus.  $\beta_6$  also contains two Arg-Gly-Asp sequences, one at amino acids 514-516 and the other at 594-596. These regions could serve as recognition sites for other ligands of the integrin family.

PCR using the primer pair B3F/B4R (see Fig. 1) amplified fragments of the expected size of approximately 750 nucleotides. Cloning and sequencing of the fragments did not result in any additional clones containing the novel  $\beta$  subunit sequence but did result in several clones with inserts encoding an amino acid sequence that was 97% identical to the corresponding region of human  $\beta_3$  and several others encoding an amino acid sequence that was 93% identical to human  $\beta_1$  (Fig. 5). These are presumably the guinea pig homologues of  $\beta_1$  and  $\beta_3$ , respectively. The nucleotide sequences of guinea pig and human  $\beta_1$  are 80% identical, and those of guinea pig and human  $\beta_3$  are 91% identical.

Figure 5 shows the alignment of partial nucleotide and amino acid sequences from Human (H) and guinea pig (GP)  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$ , and  $\beta_6$  for the region just downstream from the B3F primer. Amino acid translations denoted by the one-letter code are shown below the second



nucleotide of each codon. For the guinea pig sequences, only amino acids that differ from the human sequences are shown. The numbers shown along the right-hand margin denote the nucleotide number for human  $\beta_6$ . The sequences  
5 for human  $\beta_1$  and  $\beta_3$  are from previously published reports.

## EXAMPLE II

### $\beta_6$ Associates with $\alpha_v$ And $\alpha_f$ Subunits

To determine that the novel  $\beta$  subunit of the present invention is associated with an  $\alpha$  chain similar  
10 to other known integrins, antisera against peptides from the cytoplasmic domain sequence of  $\beta_6$  were prepared. The following amino acid peptides from the cytoplasmic sequence of  $\beta_6$  were prepared and used to immunize rabbits:  
RGSTSTFKNVTYKHR (residues 763-777) and YKHREKQKVDLSTDC  
15 (residues 774-788). The antisera were raised in rabbits according to standard procedures known in the art. Briefly, peptides were chemically coupled to keyhole limpet hemocyanin, and were injected in rabbits in either complete (first injection only) or incomplete Freund's  
20 adjuvant as described, for example, in Antibodies: A Laboratory Manual, E. Harlow and D. Lowe, eds., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724. Antisera were termed 6830 (to peptides corresponding to residues 763-777) and 6341 (to peptides  
25 corresponding to residues 774-788).

The resulting polyclonal antibodies were used to immunoprecipitate detergent lysates from the pancreatic carcinoma cell line FG-2 that had been surface radioiodinated according to procedures well known in the  
30 art such as described, for example, in Kajiji et al., EMBO J 3:673-680 (1989). A complex of two bands was precipitated of respectively 150 kilodaltons (Kd) and 97 Kd in SDS-PAGE under non-reducing conditions. Under reducing conditions, the two bands migrated as a diffused

band, extending from 130 Kd to 116 Kd. These bands were specific since pre-immune serum did not precipitate any of them and they were not present when the immunoprecipitation was carried out in the presence of the corresponding immunogenic peptide. Furthermore, the same complex of two bands was precipitated by both the 6830 and 6841 antibodies, which were raised against independent peptides from the cytoplasmic sequence deduced from  $\beta_6$  cDNA clones.

To determine which of the two precipitated bands corresponds to  $\beta_6$ , a SDS-heat denaturated lysate from surface-radioiodinated FG-2 cells was immunoprecipitated with the 6841 antibody. Only the 97 Kd band was detectable (non-reducing conditions), identifying it as the  $\beta_6$  band. Under reducing conditions, the apparent molecular weight of this band increased to 116 Kd suggesting the presence of many intra-chain disulfide bonds, which is consistent with the primary structure of  $\beta_6$  and of other integrin  $\beta$  chains.

The other band, of 150 Kd or 130 Kd under non-reducing or reducing conditions, respectively, is likely to be an  $\alpha$  subunit since it dissociates after SDS-heat denaturation of the lysate, indicating that it is non-covalently associated with the  $\beta_6$  polypeptide. Furthermore, similar to certain other integrin  $\alpha$  chains, its molecular weight decreases under reducing conditions by about 20 Kd (130 Kd versus 150 Kd under non-reducing conditions) probably due to a disulfide linked small peptide that dissociates upon reduction.

To identify which  $\alpha$  chain is associated with  $\beta_6$ , the  $\alpha\beta_6$  integrin complex was purified by immuno-affinity chromatography on a 6841-protein A sepharose matrix according to procedures well known in the art such as described, for example, in Kajiji et al., EMBO J 3:673-

680 (1989). The eluted material was immunoprecipitated with antibodies specific for  $\alpha_1$ ,  $\alpha_2$ ,  $\alpha_3$ ,  $\alpha_5$ ,  $\alpha_6$  and  $\alpha_v$ , which are known to be expressed in FG-2 cells. Only the anti- $\alpha_v$  monoclonal antibody 142.19, obtained from David Cherish, Ph.D., Scripps Clinic and Research Foundation, La Jolla, California, reacted with the purified material, which indicates that the  $\alpha_v$  is associated with  $\beta_6$  in this pancreatic carcinoma cell line.

To confirm this data, immunodepletion experiments on surface-radioiodinated FG-2 lysates were performed according to methods well known in the art such as described in Kajiji et al., EMBO J 3:673-680 (1989). The cell lysate was depleted with the 6841 anti- $\beta_6$  antibody or, in parallel, with a control antiserum, and then immunoprecipitated with the 142.19 anti- $\alpha_v$  antibody. A smaller amount of  $\alpha_v$  was present in the immunoprecipitation on the  $\beta_6$  depleted lysate and no 97 Kd  $\beta_6$  band was visible. Instead, a smaller band of about 90 Kd was present. It is hypothesized that this smaller band represents the  $\beta_5$  chain also associated with  $\alpha_v$  in these cells. In the control lysate depleted with normal rabbit serum, all three bands, 150 Kd ( $\alpha_v$ ), 97 Kd ( $\beta_6$ ) and 90 Kd ( $\beta_5$ ) were present after immunoprecipitation with the anti- $\alpha_v$  142.19 antibody.

Another immunodepletion was carried out using 142.19 antibody as the depleting antibody, or in parallel a mouse monoclonal as a control antibody. Immunoprecipitations of  $\alpha_v$ -depleted lysate with anti- $\alpha_v$  142.19 antibodies did not show the presence of any band, indicating that all  $\alpha_v$ -containing integrins had been removed. However, the 6841 anti- $\beta_6$  antibody still precipitated a complex of two bands, one corresponding to  $\beta_6$ , the other with a molecular weight close to that of  $\alpha_v$ . This  $\alpha$  chain, however, must differ from  $\alpha_v$  since it is unreactive with anti- $\alpha_v$  monoclonal antibodies and is

referred to herein as  $\alpha_f$ . In the control depleted lysates, the 6841 anti- $\beta_6$  antibody precipitates much stronger bands, consistent with the possibility that, in FG-2 cells, two  $\beta_6$  integrins exist,  $\alpha_v\beta_6$  and  $\alpha_f\beta_6$ .

- 5           Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the claims.

## WE CLAIM:

1. A substantially purified integrin cell surface receptor subunit comprising  $\beta_6$ .
2. The substantially purified integrin cell surface receptor subunit of claim 1 having the amino acid sequence set forth in Figure 3 for human.
3. A substantially purified integrin comprising  $\beta_6$  bound to an  $\alpha$  subunit.
4. The integrin of claim 3, wherein the subunit is  $\alpha_v$ .
5. The integrin of claim 3, wherein the subunit is  $\alpha_f$ .
6. A substantially purified amino acid fragment specific to  $\beta_6$ .
7. A vector comprising a gene encoding for the amino acid fragment of claim 6.
8. A host containing the vector of claim 7.
9. A reagent having specificity for an amino acid sequence specific for  $\beta_6$ .
10. The reagent of claim 9, wherein the reagent is an antibody.
11. A substantially purified nucleic acid encoding  $\beta_6$ .

12. A substantially purified nucleic acid which specifically hybridizes with a nucleotide sequence of the nucleic acid of claim 11.

13. A substantially purified nucleic acid which specifically hybridizes with the nucleic acid of claim 12 and does not hybridize with a nucleic acid encoding a non- $\beta_6$  polypeptide.

14. A method of preventing the binding of a cell expressing a  $\beta_6$ -containing integrin to ligand capable of binding to said  $\beta_6$ -containing integrin, comprising blocking the binding of the  $\beta_6$ -containing integrin and the  
5 ligand.

15. The method of claim 14, wherein the blocking is effected by binding the  $\beta_6$ -containing integrin with a reagent specific thereto.

16. The method of claim 14, wherein the blocking is effected by binding the ligand of the  $\beta_6$ -containing integrin with a reagent specific for the ligand.

17. The method of claim 15, wherein the reagent is an RGD-containing peptide or polypeptide.

18. The method of claim 15, wherein the reagent is a ligand fragment containing an integrin binding site.

19. A method of detecting a ligand that binds a  $\beta_6$ -containing integrin, comprising contacting the  $\beta_6$ -containing integrin with a solution containing the ligand suspected of binding  $\beta_6$ -containing integrins and detecting  
5 the presence of the ligand bound to the  $\beta_6$ -containing integrin.

20. A method of increasing cell adhesion in cells expressing a  $\beta_6$ -containing integrin, comprising overexpressing the  $\beta_6$ -containing integrin in a cell.

21. A method of decreasing cell adhesion in cells expressing a  $\beta_6$ -containing integrin comprising binding the  $\beta_6$ -containing integrin with a ligand.

# FIG. 1A

## CONSENSUS $\beta$

## SUBUNIT PRIMERS

$\beta_2$  human GACCTGTACTATCTGATGGACCT  
D L Y Y L M D L  
 $\beta_3$  human GACATCTACTACTTGATGGACCT  
D I Y Y L M D L  
 $\beta_1$  human GACCTCTACTACCTTATGGACCT  
D L Y Y L M D L  
 $\beta_1$  chicken GACCTTTATTATCTTATGGACCT  
D L Y Y L M D L

PRIMER B1F 5'GACCTCTACTACTCTGATGGACCT 3'  
A G T T T T

PRIMER B2R 3'CTTCCACCIAAICTACGGTAITACG 5'  
C G G T

$\beta_2$  human GGGGACTGTGTCTGCGGGCAGTGC  
G D C V C G Q C  
 $\beta_3$  human GCGAGTGCCTCTGTGTCAATGT  
G E C L C G Q C  
 $\beta_1$  human GGAGAGTGCCTGCGGACAGTGT  
G E C V C G Q C  
 $\beta_1$  chicken GGAGAGTGCATTGCGGACAGTGC  
G E C I C G Q C

PRIMER B3F 5'GGIGAITGTTTGTGGICAGTG 3'  
C C C A  
PRIMER B3R 3' CTIACAIAAACACCIGTCAC 5'  
G G G T

PRIMER B4R 3'TAACCIGAACAICGIGATIACTAIACCTT 5'  
G T GG AA A C G

$\beta_2$  human GAGGTGGGCTGGACGCCCATGATGCA  
E G G L D A M M Q  
 $\beta_3$  human GAGGTGGCTTTGATGCCCATCATGCA  
E G G F D A I M Q  
 $\beta_1$  human GAAGGTGGTTTCGATGCCCATCATGCA  
E G G F D A I M Q  
 $\beta_1$  chicken GAAGGTGGATTGATGCAATAATGCA  
E G G F D A I M Q

$\beta_2$  human ATCGGCATTCTCCTGCTGGTCATCTGGAAG  
I G I L L L V I W K  
 $\beta_3$  human ATTGGCCTTGCCGCCCTGCTCATCTGGAAA  
I G L A A L I W K  
 $\beta_1$  human ATTGGCCTTGCACTTACTGCTGATATGGAAG  
I G L A L L I W K  
 $\beta_1$  chicken ATTGGACTTGCACTTGTATTGATTGGAAA  
I G L A L L I W K



2/11

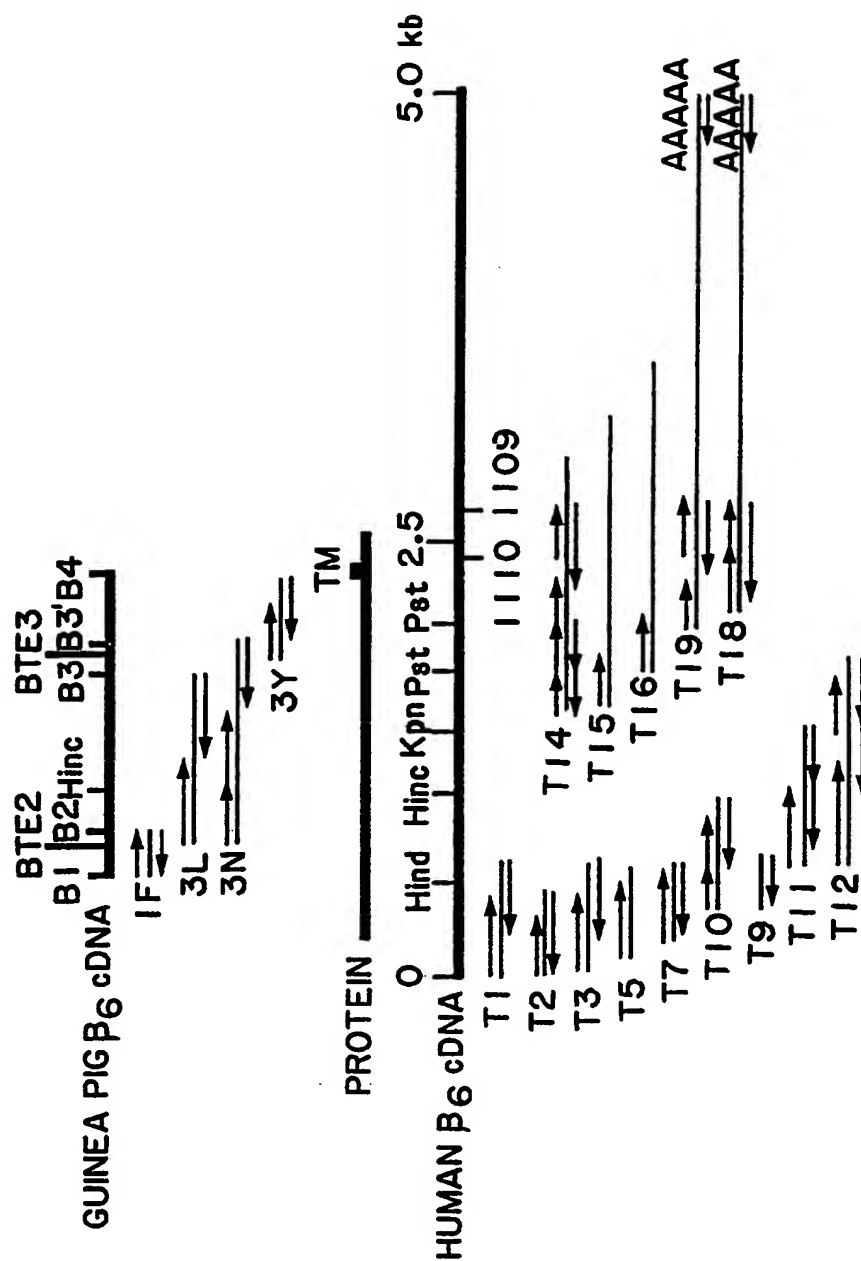
**β<sub>6</sub> PRIMERS**

β <sub>6</sub> guinea pig	nt 219	CCATTGACAAATGATGCTGAAAGA
		P L T N D A E R
<u>PRIMER BTE2F</u>		5'CCITTITACIAATGATGCIGAAAGA 3'
		C C
β <sub>6</sub> guinea pig	1325	CATCTCCGAAGACGGCA
		I S E D G
<u>PRIMER BTE3F</u>		5'CATCTCCGAAGACGGCA 3'

**FIG. 1B**SUBSTITUTE SHEET

3/11

FIG. 2



SUBSTITUTE SHEET

4 / 11

B6HUMAN TAAACACAGCTTTTCTGCTTTACCTGTCCAGGTAGCCTCTGTTTTTATT  
 B6H AGTGTAAGTAGTATTTAAAATGTTATACTTCAAGAAAGAAAGACTTTAACG  
 B6H CTCGCACAGCAAGAACTGAAACGAATGGGGATTGAACTGCTTTGCCTGTTC  
 B6H GGTGCAGAAACCTGTGAAGACTGCCTGCTTATTGGACCTCAGTGTGCCTGG  
 B6H ACCCCAGCAAACCTTTTAGCTAAAGGATGTCAATTAACTTCATCGAAAC  
 B6H CAGAAAAATAGTTCTGACATTGTTTCAAGATTGCACCTCAAAGCTTGATCCTT  
 B6H GAGGACTACCCGGTGGATTGTATTACCTCATGGACCTCTCCGCCTCCATG  
 B6GUINEA PIG TCCGCCTCCATG  
 B6H ATGTCTAAATTAACCAGCAACTTTAGACTGGGCTTCGGATCTTTTGTGGAA  
 B6GP ATGTCTAAATTAAGTAGCAACTTTAGACTGGGCTTCGGCTCTTTTGTAGAA  
 B6H TGCAGTAGTATTCCATACTTCTGTTTACCTACATTTGGATTCAAGCACATT  
 B6GP TGCAGTAGTATTCCATATATCTGCTTACCTACATTTGGATTCAAGCACATT  
 B6H AAAATTTCTGCTAATATTGACACACCCGAAGGTGGATTTGATGCAATTATG  
 B6GP AAAATTTCTGCTAATATTGACAACCCTGAAGGTGGATTCGACGCCATTATG  
 B6H CTCCTGGTCTTTGTGAGTGATGCTGATTCTCATTTTGGAAATGGACAGCAAA  
 B6GP CTCCTAGTCTTCGTGAGTGATGCCGATTCTCATTTTGGAAATGGACAGCAAA  
 B6H AATGAATACTCCATGTCAACTGTCTTGAATATCCAACAATTGGACAACCTC  
 B6GP AATGAATACTCCATGTCAACTGTGATGGAATATCCAACAATTGGACAACCTC  
 B6H GAACAAGTTCATTTATATGAGAATTACGCAAACTTATTCCTGGAGCTACA  
 B6GP GAACAAGTTCACCTATATGAGAATTATGCAAACTTATTCCTGGAGCCACA  
 B6H GCTTATGAAGAACTGCGGTCTGAGGTGGAAGTATTAGGAGACACT  
 B6GP GCTTATGAAGAACTGCGGTCTGAGGTGGAAGTATTAGGAGATACA

FIG. 3A

SUBSTITUTE SHEET

5/11

TCAGTCTTAATGAAAACCTTTCTAACTTATATCTCAAGTTTCTTTTCAAAGC 100  
 ATATTCAGCGTTGGTCTTGTAACGCTGAAGGTAATTCATTTTTTAATCGGT 202  
 TTTCTATTTCTAGGAAGGAATGATTCACGTACAAGGTGGCTGTGCCTGGGA 304  
 F L F L G R N D S R T R W L C L G 26  
 TGTGCTCAGGAGAATTTTACTCATCCATCTGGAGTTGGCGAAAGGTGTGAT 406  
 C A Q E N F T H P S G V G E R C D 60  
 CCTGTCTCCCAAGTAGAAATACTTAAAAATAAGCCTCTCAGTGTAGGCAGA 508  
 P V S Q V E I L K N K P L S V G R 94  
 AAGTTGAGACCAGGTGGTGGCAGACTCTGCAGGTGCATGTCCGCCAGACT 610  
 K L R P G G A Q T L Q V H V R Q T 128  
 GATGACGACCTCAACACAATAAAGGAGCTGGGCTCCGGCCTTTCCAAAGAG 712  
 D D D L N T I K E L G S G L S K E 162  
 GACGATGACCTCAACACAATCAAAGAGCTGGGCTCCCTGCTTTCAAAGGAG 63  
 L  
 AACCTGTATCCCTTTTGTGAAAACAACACCAGAAGAAATTGCCAACCT 814  
 K P V S P F V K T T P E E I A N P 196  
 AAACCGTCTCCCTTTTATGAAAACAACACCAGAGGAAATTGCCAACCT 165  
 M 55  
 TTGCCATTGACAAATGATGCTGAAAGATTCAATGAAATTGTGAAGAATCAG 916  
 L P L T N D A E R F N E I V K N Q 230  
 CTGCCATTGACAAATGATGCTGAAAGATTCAATGAAATTGTGAAGAAACAG 267  
 89  
 CAAGCTGCTGTGTGTAAGGAAAAAATTGGCTGGCGGAATGACTCCCTCCAC 1018  
 Q A A V C K E K I G W R N D S L H 264  
 CAAGCTGCTGTGTGTAAGGAAAAAATTGGCTGGCGGAATGATTGCTCCAT 369  
 123  
 CTAGCAGGCATCGTCATTCTAATGACGGGCTCTGTCACTTGGACAGCAAG 1120  
 L A G I V I P N D G L C H L D S K 298  
 CTGGCAGGCATTGTCATTCCCAACGATGGGCTGTGTCACTTGGACAGCAAG 471  
 157  
 ATTGATAAACTGGTACAAAACAACGTGTTATTGATCTTCGCTGTAACCCAA 1222  
 I D K L V Q N N V L L I F A V T Q 332  
 ATTGATAAAGTGGTACAAAACAATGTGTTACTGATCTTTGCTGTAACCCAA 573  
 191  
 GTAGGTCTACTTCAGAAGGACTCCGGAACATTCTCCAGCTGATCATCTCA 1324  
 V G L L Q K D S G N I L Q L I I S 366  
 GTGGGGCTACTTCACAAGGACTCTGGAACATTCTCCAAGTATCATCTCA 675  
 H 225  
 GAAGGACTCAACTTGTCAATTTACAGCCATCTGTAACAACGGTACCCTCTTC 1426  
 E G L N L S F T A I C N N G T L F 400  
 GAGGGCCTCAATCTTTCGTTCTCAGCTGTCTGTAACAATGGCACTCTCTTC 777  
 259

FIG. 3B

SUBSTITUTE SHEET

6/11

B6H CAACACCAAAGAAATGCTCTCACATGAAAGTGGGAGACACAGCTTCCTTC  
       Q H P K K C S H M K V G D T A S F  
 B6GP CCACACCAAAGAAATGCTTGCACATGAAAGTGGGAGAAACAGCTTCATTC  
       P L E  
 B6H ATAAAGCCTGTGGGGCTGGGGGATGCCCTGGAATTACTTGTGAGCCAGAA  
       I K P V G L G D A L E L L V S P E  
 B6GP ATAAAGCCTGTGGGGCTGGGGGACACCCTGGAAATCCTTGTGAGCCAGAA  
       T I  
 B6H CACGGGAACGGCTCTTTCCAGTGTGGGGTGTGTGCCTGCCACCCTGGCCAC  
       H G N G S F Q C G V C A C H P G H  
 B6GP AATGGGAACGGCTCTTACCAGTGTGGGGTGTGTGCCTGTAACCCAGGCCAC  
       N Y N  
 B6H AAGGAGGCCCCAGATCATCCCTCCTGCAGCGGAAGGGGTGACTGCTACTGT  
       K E A P D H P S C S G R G D C Y C  
 B6GP AAGGAGACCCAGACCATCCCTCGTGCAGCGGAAGGGGTGACTGCTACTGT  
       T  
 B6H TGCCAGTGTGACAATTTCTCCTGCGTGAGACACAAAGGGCTGCTCTGCGGA  
       C Q C D N F S C V R H K G L L C G  
 B6GP TGCCAGTGTGACAATTTCTCCTGTGTGAGGCACAAAGGGCTGCTCTGTGGA  
       T  
 B6H GGCGAGTACTGCAACTGCACCACCAGCACGGACTCCTGCGTCTCTGAAGAT  
       G E Y C N C T T S T D S C V S E D  
 B6GP GGAGAGTACTGCAACTGTACCACCAGCACAGACACTGCATCTCCGAAGAC  
       T I  
 B6H ACAAACCCTGGAGCCTCAGGACCAADCTGTGAACGATGTCCTACCTGTGGT  
       T N P G A S G P T C E R C P T C G  
 B6GP ACGAACCCTGGAGCCTCGGGACCCACCTGTGAACGATGTCCTACCTGTAGT  
       P V  
 B6H GGCCAAGCCGGAGAAGAATGTGTGGACAAGTGCAAAGTGGTGGGACC  
       G Q A G E E C V D K C K L A G A T  
 B6GP GGTCAGCCTGGAGAAGAATGTGTGGACAATGCAAAGTGGTGGGACC  
       P V  
 B6H CAAGGAGAAAATGAATGTTTAATTACATTCTAATAACTACAGATAATGAG  
       Q G E N E C L I T F L I T T D N E  
 B6GP CAAGGAGAAAATGAATGTCTTATTACATTCTAATAAGTACAGATAATGAG  
       S  
 B6H AACATTCCCATGATCATGTTAGGGGTTTCCCTGGCTACTCTTCTCATCGGG  
       N I P M I M L G V S L A T L L I G  
 B6GP AATATTCTATGATCATGTTGGGGGTTTCACTGGCTA  
       S  
 B6H GAAGTTGCCAAATTTGAAGCAGAACGATCAAAGCCAAGTGGCAAACGGGA  
       E V A K F E A E R S K A K W Q T G  
 B6H AAACACAGGGAAAAACAAAAGGTAGACCTTTCCACAGATTGCTAGAACTAC  
       K H R E K Q K V D L S T D C

FIG. 3C

SUBSTITUTE SHEET

7/11

AGCGTGACTGTGAATATCCCACTGCGAGAGAAGAAGCAGGCACATTATC 1528  
 S V T V N I P H C E R R S R H I I 434  
 AATGTGACTGTGAGTATACCAAAGTGTGAGAGAAAAAGCAGGCATGTTATC 879  
 N S N K V 293  
 TGCAACTGCGACTGTCAGAAAGAAGTGAAGTGAACAGCTCCAAATGTCAC 1630  
 C N C D C Q K E V E V N S S K C H 468  
 TGCAGCTGCGATTGTCAGAAAGAAGTGAAGTGAACAGCTCCAAATGCCAC 981  
 S 327  
 ATGGGGCCTCGCTGTGAGTGTGGCGAGGACATGCTGAGCACAGATTCTCTGC 1732  
 M G P R C E C G E D M L S T D S C 502  
 ATGGGGCCTCACTGCGAGTGTGGTGAAGACACGCTGAGCACAGATTCTCTGC 1083  
 H 361  
 GGGCAGTGTATCTGCCACTTGTCTCCCTATGGAAACATTTATGGACCTTAT 1834  
 G Q C I C H L S P Y G N I Y G P Y 536  
 GGGCAGTGCATCTGCCACTTGTCTCCCTATGGAAACATTTATGGACCTTAC 1185  
 395  
 GGTAAACGGCGACTGTGACTGTGGTGAATGTGTGTGCAGGAGCGGCTGGACT 1936  
 G N G D C D C G E C V C R S G W T 570  
 GATAACGGAGACTGTGAATGTGGGAATGCGTGTGCAGGAGTGGTTGGACC 1287  
 D E 429  
 GGAGTGCTCTGCAGCGGGCGGGGACTGTGTTTGTGGCAAGTGTGTTTGC 2038  
 G V L C S G R G D C V C G K C V C 604  
 GGCAGGCTCTGCAGCGGGCGGGGACTGCGTCTGTGGCAAGTGTGTCTGC 1389  
 T 463  
 GACCCCTGTAACCTAAACGGAGCTGCATTGAGTGCCACCTGTCAGCAGCT 2140  
 D P C N S K R S C I E C H L S A A 638  
 GACCCCTGTAACCTAAACGGAGCTGCATTGAATGCCACCTGTCTGCAGAT 1491  
 S D 497  
 ATCAGTGAAGAAGAAGATTTCTCAAAGGATGGTTCTGTTTCCTGCTCTCTG 2242  
 I S E E E D F S K D G S V S C S L 672  
 ATCAGCAAAGAAGCAGATTTCTCAAAGGATAGTTCTGTTTCCTGCTCCCTG 1593  
 K A S 531  
 GGGAAAACCATCATTACAGCATCAATGAAAAAGATTGTCCGAAGCCTCCA 2344  
 G K T I I H S I N E K D C P K P P 706  
 GGAAAAACCATCATTACAACATCAGTGAAGAAAGACTGCCCCAACCTCCA 1695  
 N S 565  
 GTTGTCTACTGTGCATCTGGAAGCTACTGGTGTCAATTCATGATCGTAAA 2446  
 V V L L C I W K L L V S F H D R K 740  
 1732  
 ACCAATCCACTCTACAGAGGATCCACAAGTACTTTTAAAAATGTAACCTAT 2548  
 T N P L Y R G S T S T F K N V T Y 774  
 TTTATGCATAAAAAAGTCTGTTTCACTGATATGAAATGTTAATG 2644  
 788

FIG. 3D

SUBSTITUTE SHEET

FIG. 4A

B1	human	MNLOPIFWIGLISSVCCVFAQT	
B2	human	MLGLRPPPLIALVGLLSLGCV	
B3	human	MRARPRRPLWVTVLALGALAGVG	
B <sub>myo</sub>	Drosophila	MILERNRRCQLALLMIAMLAIAAQTNAQKAALIT	
B6	human	MGIELLCLEFFFLGRNDSR	
B1		DENRCLKANAKSCGEIC <sup>*</sup> IOAGPNCG <sup>*</sup> WCTNSTFFOEGMPTSA <sup>*</sup> RCDDLEALKKKGCPDDIEN <sup>*</sup> RGSKDIKKNKVNTRSKGTAEKLPEDIHQ	112
B2		LSQECTKFKVSSCRECIESGPGCTWCQKLNFTGPGDPDSIRCDTRPQLLMRGCAADDIMDPTSLAETQEDHNGGQK	QLSPQK 101
B3		GPNIC <sup>*</sup> TRGVSSCQQCLAVSPMCWCSD <sup>*</sup> DEAL PLGSPRCDLKENLLKDNCAPE <sup>*</sup> IEFPVSEARVLEDRPLSDKSGDSSQVTVQVSPQR	112
B <sub>myo</sub>		AVSTCA SKEKCHTCIQTE GC <sup>*</sup> AWCMQPDF KGQS RC Y QN TSSLCPEEFAYSPIITVEQILVNNKLTNQYKAE insert	IVQ 137
B6		TRWLC LG GAETCEDCLLIGPQCAWCAQENFTHPSGVGE RCDTPANLLAKGCQLNFIE <sup>*</sup> NPVSQVEILKNKPLSVGRQKNSS	DIVQ 103
B1		IQQQLVLR <sup>*</sup> LRSGEPQFTLKF <sup>*</sup> KRAEDY <sup>*</sup> PI <sup>*</sup> DL <sup>*</sup> YYIM <sup>*</sup> DL <sup>*</sup> SYSM <sup>*</sup> KDD <sup>*</sup> LENVKS <sup>*</sup> L <sup>*</sup> GTD <sup>*</sup> LMNEMRRRI <sup>*</sup> SDFRIG <sup>*</sup> SG <sup>*</sup> FVE <sup>*</sup> K <sup>*</sup> TM <sup>*</sup> Y <sup>*</sup> ISTT <sup>*</sup> EAK	L 202
B2		VT LYL <sup>*</sup> RPGQAAAFNVTF <sup>*</sup> RAKGY <sup>*</sup> PI <sup>*</sup> DL <sup>*</sup> YYIM <sup>*</sup> DL <sup>*</sup> SYSM <sup>*</sup> LDD <sup>*</sup> LRNVKKLG <sup>*</sup> DL <sup>*</sup> LRALNEITESGRIG <sup>*</sup> SG <sup>*</sup> FVDK <sup>*</sup> TVLPFV <sup>*</sup> THPDK	L 186
B3		IA LRL <sup>*</sup> RDDSKNFSIQVRQVEDY <sup>*</sup> PVDI <sup>*</sup> YYIM <sup>*</sup> DL <sup>*</sup> SYSM <sup>*</sup> KDD <sup>*</sup> LWSIQNLG <sup>*</sup> TKLATQMRKLT <sup>*</sup> SNLRIG <sup>*</sup> GAFVDK <sup>*</sup> PVSPYMYIS <sup>*</sup> PEAL	198
B <sub>myo</sub>		IQPQSMRLALRVNEKHNIKISYSQAEGYPVDLYYLMDLSKSMEDDKAKLSTLGD <sup>*</sup> KLSETMKRITNNFHLG <sup>*</sup> SGFVDK <sup>*</sup> VLMPYVSTIPKK	L 227
B6		IAPQSLILKLRPGGAQT <sup>*</sup> LQVHVRQTEDY <sup>*</sup> PVDLYYLMDLSASMD <sup>*</sup> DD <sup>*</sup> INTIKELG <sup>*</sup> SLSKEMSKLT <sup>*</sup> SNFRIG <sup>*</sup> SGFVEK <sup>*</sup> PVSPFVKTTPEE	I 193
B1		RN <sup>*</sup> FC TSEQNCTTPFSYKNVLS <sup>*</sup> LTNKG <sup>*</sup> EV <sup>*</sup> FNEL <sup>*</sup> VGKQRI <sup>*</sup> SGN <sup>*</sup> L <sup>*</sup> DS <sup>*</sup> PE <sup>*</sup> GG <sup>*</sup> FD <sup>*</sup> AIM <sup>*</sup> QVAV <sup>*</sup> CGSLI <sup>*</sup> GN <sup>*</sup> VTRL <sup>*</sup> LV <sup>*</sup> ST <sup>*</sup> DAG <sup>*</sup> F <sup>*</sup> H <sup>*</sup> FAG <sup>*</sup> D <sup>*</sup> GK <sup>*</sup> LG	291
B2		RN <sup>*</sup> PCPNKEKECQPPFAFRHVLKLTNNSNQ <sup>*</sup> FQTEVGKQLISGNLDAPEGG <sup>*</sup> LDAMQVAA <sup>*</sup> CPEEIGWRN VTRL <sup>*</sup> LV <sup>*</sup> FATDDG <sup>*</sup> FH <sup>*</sup> FAGD <sup>*</sup> GK <sup>*</sup> LG	276
B3		ENPCYDMKTTCLPMFGYKHVLT <sup>*</sup> LDQVTRFNEEVKKQSVSRNRDAPEGG <sup>*</sup> FD <sup>*</sup> AIMQATV <sup>*</sup> CDEKIGWRNDASHLLV <sup>*</sup> FTTDAK <sup>*</sup> THIALD <sup>*</sup> GRLAG	289
B <sub>myo</sub>		EHPC ENCKAPYGYQN <sup>*</sup> HMP <sup>*</sup> LNNNTESF <sup>*</sup> SN <sup>*</sup> EVKNATV <sup>*</sup> SGNLD <sup>*</sup> PEGG <sup>*</sup> FD <sup>*</sup> AIMQAIACRSQIGWRNQARLLV <sup>*</sup> FTSDAG <sup>*</sup> FH <sup>*</sup> YAGD <sup>*</sup> GK <sup>*</sup> LG	314
B6		ANPCSSIPYFC <sup>*</sup> LPTFGFKHILPLTND <sup>*</sup> AERFNEIVKNQKISANIDTPEGG <sup>*</sup> FD <sup>*</sup> AIMQAAV <sup>*</sup> CKEIKIGWRNDSLHLLV <sup>*</sup> FTSDADSHFGMDSKLAG	284

FIG. 4B

B1	IVLPNDGQCHLENNM	YTMŠHYDYPSIAHLVQKLENNNIQTI	FAVTEEFQPVYKELKNLIPKSAVGTLSANŠŠNVIQIIDAŲNLSSEV	381					
B2	ILTPNDGRCHLEDNL	YKRSNEFDYPSVQLAHKLAEENNIQPIFAVTSRMVKTYEKLTEI	IPKSAVGELSEDSŠNVVHLIKNAYNKLSSRV	366					
B3	IVQPDGQCHVGSNDHYSASTMDYPSLGLMTEKLSQKNINLIFAVTENVNLYQNYSELIPGTTVGVLŠMDSSNVLQLIIVDAYGKIRSKV	380							
Bmyo	VIAPNDGECHLSPKGEYTHSTLQDYPSISQINQKVKDNAINIIFAVTASQLSVYEKLVVEHIQGSAAKLDNDSSNVVELVKEEYRKISSSV	405							
B6	IVIPNDGLCHLDSKNEYSMTVLEYPTIGQLIDKLQNNVLLIFAVTQEQVHLYENYAKLIPGATVGLLQKDSGNILQLIISAYEELRSEV	375							
B1	ILENGKLSEGV	ISYKSYCKNGVNGTGENGGRKCSNISIGDEVQFEISITSNKCPKK	D	SDSFKIRPLGFTTEEVEVILQYICECECQSEG	469				
B2	FLOHNALPDTLKV	YDSFCSNGVTHRNQPRGDCDGVQINVPITFQVKVTATECIEQ	Q	SFVIRALGFTDIVTVQVLPQCECRRDQS	452				
B3	ELEVRLPEELSLSFNATCLNNEVIPGL	KSCMGLKIGDTSFSIEAKVRGCPQE	K	EKSFTIKPVGFKDSLIVQVTFDCDCACQAQA	466				
Bmyo	EMKDNATGD	VKITYSFSSCLSNGPVQV	SKCDNLKEGQVSTFAQIQLLKCPEPRDW	TQTIHISPVGINEVMQIQLTMLCSCPCENPG	493				
B6	ELEVIGDTEGLNLSFTAICNNGTLFQHP	KKCSHMKVGD	TASFSTVNIPHC	ER	R	SRHIIKIPVGLGDALELLVSPENCDCQKEV	460		
B1	I	PESPKCHEGNGTFECGACRCNEGRVGRHCECSTDEVN	ŠEDM	DAYCRKENSS	EICŠNNGEČVCČQČVČKCRKRDNTNEIYSČKFČE	553			
B2	R	DRSLCH	GKGFLECGICRCDTGYIGKNCECQTOGRS	SOEL	EGSCRKDNN	IICŠGLGDCVCGQCLCHTSDVPGLIYGQYCE	534		
B3	E	PNSHRCNNGNGTFFECGVCRGPGWLGSCQCECSEEDYRPSQ	Q	DE	CSPREGQ	PVCSQRGECLCGQCVCHSSDF	GKIT	GKYCE	547
Bmyo	SIGYQVQANSCS	GHGTSMCGICNCDDSYFGNKECSATDLT	SKFANDTSCRADSTST	TDCSGRGHCVCGACECHKRPNPIE	IISGKHCE	582			
B6	E	VNSSKCHGNGSFQCGVCACHPGHMGPRCEGEDML	ST	D	SCKEAPDH	PSCSGRGDCYCGQCICHLSPY	GN	IYGPYQ	538

-----B3-----



10/11

B1	*CDNFNCDRNSGLICGG	*NGVCKCRVCECNPNYTGSA	*CDSCSLDSTCEASN	*GOICNGRGICCEGVCCKT	*DPKFOGQTCEMCQTCLGV	638
B2	CDTINCERYNGQVCGGPG	GLCFGCKRCHPGFEGSACQ	CERTTEGCLNPR	RVECSGRGRRCNVCCH	SG YQLPLCQECPCPSP	620
B3	CDDFSCVRYKGEPCSG	HGQSCGDCCLCDSDWTG	YGCNCTTRDT	TCMSSN GLLCSGRGKCEG	SCVCI	632
Bmyo	CDNFSCERNRNQLCSPD	HGTCEGRCCKCPGWTG	SNCGCQESNDTCMP	PGGGEICSGHGTCEGVCCKT	VNDQGRFSGRHCCKPTCSGR	673
B6	CDNFSCVRHKGLLCGG	NGDCDCGEGCVCRSGWT	GEYCNCTTSTDSCVSED	GVLCSSGRGDCVCGKCVCT	NPASGPTCERCPTCGDP	623
B1	*CAEHKECVQCRAFNKG	*KKDTCTQECYSYFNITK	VESRDKLQPVQPD	PVSHCKEKEVDVDDCNW	*FYFTY SVNGNNEVMHV	VENPECTGP 726
B2	CGKYISCAECLKFEKGP	F GNCSAACPG	LQLSN	NPVKGR	T CKERDSEGCWVAYTLE	QQQDMDRYLIYVDESRECVAGP 698
B3	CTFKKECCECKKFDRE	PYMTENTCNRYCRDE	IESVKELKD	TGKDAVN	CTYKNEDDCVVR	FQY YEDSSGKSILYVVEEPCPKGP 715
Bmyo	CQELKDCVQCQMYKT	GELKNGDDCARNCTQ	FVPVGVKEVEID	ETKDEQM	CKFFEDDDCKFMFY	SEQGELHVYAQENKECPAKV 757
B6	CNSKRSCIECHLSAAG	QA GEECVDKCKLAGA	TISEEEEDF	SKDGSVS	CSLQGENECLITFLI	TTDNEGKTIHSINEKDCPKPP 706
B1	DIIPVAGVAGIVLIGL	ALLIWLMIHRRREFA	FEKEKMNNAKNDT	GENPIYKSAVT	TVNPKYEGK	797
B2	NIAAIVGGTVAGIVL	IGILLVWIKALIHLS	DLREYRFEKEKLSQ	WNN DNFLFKSAT	TTVMNPKFAES	769
B3	DILVLLSVMGAILL	IGLALLWIKLLITI	HDRKEFAKFEERARAK	WDTANNPLYKEAT	STFTNITYRGT	786
Bmyo	FMLGIVMGVIAAIV	LVGLAILLWIKLLT	TIHRRREFARFEKER	MNAKWDGENPIYKQAT	STTFKNPMYAGK	828
B6	NIPMIMLGVS	LATLLIGVLLCIVK	LLVSFHDRKEVAKF	EAERSKAKWQGTGN	PLYRGSTSTFKNVTYKHREKQKVDLSTDC	788

FIG. 4C

11/11

β1H TGTGTTGTAGGAAGAGGGATATAACAATGAAATTTATTCTGGCAATTTCTGGAGTGTGATAATTTCAACTGTGATAGATCCAAATGGCTTAATT  
 C V C R K R D N T N E I Y S G K F C E C D N F N C D R S N G L I  
 β1GP TCGGTGTGCAGGAAGAGGGACACACCAACAGAGATCTACTCTCGGCAATTTCTGGAGTGCAGACAATTTCAACTGTGATCGGTCCAATGGCTTAATC  
 β3H TGTGCTGCCACAGCAGTGACTTT GGCAAGATCAGG G K Y C E C D D F S C V R Y K G E M  
 C V C H S S D F G K I T  
 β3GP TGTCTCTGCCACAGCGATGACTTT GGCAAGATCAGG GGCAAGTACTGTGAGTGTGATGACTTCTCTGTGTTCTGCTACAAAGGGGAGATG  
 β6H TGTATCTGCCACTTGTCTCCCTAT GGAAACATTTAT G P Y C Q C D N F S C V R H K G L L  
 C I C H L S P Y G N I Y  
 β6GP TGCATCTGCCACTTGTCTCCCTAT GGAAACATTTAT GGACCTTACTGCCAGTGTGACAATTTCTCTGTGAGGCACAAAGGGCTGCTC 1888  
 β1H TGTGGAGGAATGGTGTGTTGCCAAGTGTGTTGAGTGCAACCCCAACTACACTGGCAGTGCATGTGACTGTTCTTTGGATAGTACTTGT  
 C G G N G V C K C R V C E C N P N Y T G S A C D C S L D T S T C  
 β1GP TGTGGAGGAATGGAGTGTGCGGTGTGTTGCGAGTGTCTCCCAACTACACCGGCGAGCTGTGACTGTCTCTTGACACTGCGCGCTGC  
 β3H TGTCTAGGCCATGGCCAGTGCAGCTGTGGGACTGCCCTGTGTGACTCCGACTGGACCGGCTACTACTGCAACTGTACCACGCGTACTGACACCTGC  
 C S G H G Q C S C G D C L C D S D W T G Y Y C N C T T R T D T C  
 β3GP TGTCTAGGCCATGGCCAGTGCAGCTGTGGGACTGCCCTGTGTGACTCCGACTGGCTACTACTGTAACTGTACCACACTTCACTGACACCTGC  
 β6H TGTGGAGGTAACGGCGACTGTGACTGTGTTGAAATGTGTGTCAGGAGCGGCTGGAGTGCAGAGTACTGCAACTGCACCCACGACGACTCCTGC 1984  
 C G G N G D C D C G E C V C R S G W T G E Y C N C T T S T D S C  
 β6GP TGTGGAGTAACGGAGACTGTGAAATGTGGGAATGCGTGTGCAGGAGTGTGTTGGACCGGAGAGTACTGCAACTGTACCACGACGACACACCTGC  
 β1H GAAGCCAGCAACGGACAGATCTGCAATGGCCGGGCATCTCGGAGTGTGGTCTGTGTAAGTGTACAGATCCGAAAGTTTCAAGGGCAACG  
 E A S N G Q I C N G R G I C E C G V C K C T D P K F Q G Q T  
 β1GP CTGGCCACCAACGGGCAGATCTGCAATGGCCGGGTGTGTCGAGTGCAGTGCAGGACCCCAAGTTCCAGGGGCGAGCC  
 β3H ATGTCCAGCAATGGGCTGTGTGTCAGCGCGCGGCAAGTGTGAATGTGGCAGTTGTGTCTGTCATCCAGCCGGGATCTTTATGGGGACACT  
 M S S N G L L C S G R G K C E C G S C V C I Q P G S Y G D T  
 β3GP ATGTCCAGCAACGGGCTGTGTGTCAGCGCGCGGCAAGTGTGAATGTGGCAGTTGTGTCTGTCATCCAGCCGGGATCTTTATGGGGACACT  
 β6H GTCTCTGAAGATGGAGTGTCTGTGAGCGGGGACTGTGTTGTGGCAAGTGTGTTGTGCAACACCTGGAGCCTCAGGACCAACC  
 V S E D G V L C S G R G D C V C G K C V C T N P G A S G P T  
 β6GP ATCTCCGAAGACGGCACCGCTCTGTGAGCGGGGCGGGGACTCGCTGTGTGCGCAAGTGTGTCTGTGACGAAACCTTGGAGCCTCGGGACCCACC

FIG. 5

SUBSTITUTE SHEET

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US91/00236

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) * According to International Patent Classification (IPC) or to both National Classification and IPC IPC(5): C12N 5/10, 15/09, 15/11, 15/12, 15/03, 15/07; G01N 33/566; C07K 15/06, 15/14, 15/24, 15/28 US Cl: 530/350, 395, 387; 536/27; 435/320.1, 252.3, 240.2, 69.1; 436/503						
<b>II. FIELDS SEARCHED</b> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Minimum Documentation Searched *</div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 30%; border-bottom: 1px solid black;">Classification System</th> <th style="border-bottom: 1px solid black;">Classification Symbols</th> </tr> <tr> <td style="padding: 5px;">U.S. Cl.</td> <td style="padding: 5px;">530/350, 395, 387; 536/27; 435/320.1, 252.3, 240.2; 69.1; 436/503</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched *</div> <p style="padding: 5px;">APS and DIALOG Files 155,5,399, WPI, 35, 340 and 357 for integrin and receptor and (B6 or beta 6) and N-terminal sequences.</p>			Classification System	Classification Symbols	U.S. Cl.	530/350, 395, 387; 536/27; 435/320.1, 252.3, 240.2; 69.1; 436/503
Classification System	Classification Symbols					
U.S. Cl.	530/350, 395, 387; 536/27; 435/320.1, 252.3, 240.2; 69.1; 436/503					
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT</b> **						
Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages **	Relevant to Claim No. **				
<u>X</u> <u>Y</u>	The Journal of Biological Chemistry. Vol. 265. No. 20. issued 15 July 1990. Sheppard et al. "Complete Amino Acid Sequence of a Novel integrin B Subunit (B6) identified in Epithelial Cells Using the Polymerase Chain Reaction. pages 11502-11507. See whole publication; especially the abstract and p. 11505 and 11506.	<u>1-13</u> <u>1-21</u>				
<u>X</u> <u>Y</u>	The EMBO Journal. Vol. 8. No 10. issued 1989. Freed et al.. "A Novel integrin Beta subunit is Associated with the Vitronectin Receptor Alpha Subunit (alpha) is a Human Osteosarcoma Cell Line and is a Substrate for Protein Kinase C". pages 2955-2965. See whole publication; especially the abstract.	<u>1-5</u> <u>1-21</u>				
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: **</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div> </div>						
<b>IV. CERTIFICATION</b>						
Date of the Actual Completion of the International Search *  <div style="text-align: center; font-weight: bold;">23 APRIL 1991</div>	Date of Mailing of this International Search Report *  <div style="text-align: center; font-weight: bold;">22 MAY 1991</div>					
International Searching Authority *  <div style="text-align: center;">ISA/US</div>	Signature of Authorized Officer ** <div style="display: flex; align-items: center;"> <div style="flex: 1;"> <p style="text-align: center;">NGUYEN T. C. HO INTERNATIONAL DIVISION Keith C. Furman</p> </div> <div style="flex: 1; text-align: right;"> </div> </div>					

## III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Cell . Vol. 44. Issued 28 February 1986. Ruoslahti et al. Arg-Gly-Asp: A Versatile Cell Recognition Signal". pages 517 and 518.	14-21